A Mitochondrial Approach to Cardiovascular Risk and Disease

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Abstract

Cardiovascular diseases (CVDs) are a leading risk factor for mortality worldwide and the number of CVDs victims is predicted to rise through 2030. While several external parameters (genetic, behavioral, environmental and physiological) contribute to cardiovascular morbidity and mortality; intrinsic metabolic and functional determinants such as insulin resistance, hyperglycemia, inflammation, high blood pressure and dyslipidemia are considered to be dominant factors. High cardiac energy demand is sustained by mitochondrial ATP production, and abnormal mitochondrial function has been associated with several lifestyle- and aging-related pathologies in the developed world such as diabetes, non-alcoholic fatty liver disease (NAFLD) and kidney diseases, that in turn can lead to cardiac injury. In order to delay cardiac mitochondrial dysfunction in the context of cardiovascular risk, regular physical activity has been shown to improve mitochondrial parameters and myocardial tolerance to ischemia-reperfusion (IR). Furthermore, pharmacological interventions can prevent the risk of CVDs. Therapeutic agents that can target mitochondria, decreasing ROS production and improve its function have been intensively researched. One example is the mitochondria-targeted antioxidant $MitoQ_{10}$, which already showed beneficial effects in hypertensive rat models. Carvedilol or antidiabetic drugs also showed protective effects by preventing cardiac mitochondrial oxidative damage. This review highlights the role of mitochondrial dysfunction in CVDs, also show-casing several approaches that act by improving mitochondrial function in the heart, contributing to decrease some of the risk factors associated to CVDs.

Abbreviations

4HNE – 4-hydroxy-2-nonenal

ACE – Angiotensin-converting-enzyme

ADP – Adenosine Diphosphate

ADQI – Acute Dialysis Quality Initiative

AGEs – Advanced Glycation End Products

ATP – Adenosine Triphosphate

BMI – Body Mass Index

CD36 – Cluster of Differentiation 36

CER – Ceramides

cGMP – Cyclic GMP

CKD – Chronic Kidney Disease

CL – Cardiolipin

CoQ10 – Co-enzyme Q10

CPT – Carnitine Palmitoyltransferases

CVDs – Cardiovascular Diseases

DAG – Diacylglycerol

DM – Diabetes Mellitus

Drp1 – Dynamin-related Protein

ECFCs – Endothelial Colony-forming Cells

ECM – Extracellular Matrix

eNOS – Endothelial Nitric Oxide Synthase

ETC – Electron Transport Chain

FAO – Fatty Acid β-oxidation

FFA – Free Fatty Acids

GLUT4 – Glucose Transporter type 4

HDL – High Density Lipoprotein

HDL-C – High-density Lipoprotein Cholesterol

HF – Heart Failure

HFD – High-fat Diet

Hsp – Heat Shock Protein

HUVECs – Human Umbilical Vein Endothelial Cells

IHD – Ischemic Heart Disease

IMM – Inner Mitochondrial Membrane

IR – Ischemia-reperfusion

MAPK – Mitogen-activated Protein Kinase

MCU – Mitochondrial Ca2+ Uniporter

MFN – Mitofusin

MI – Myocardial Ischemia

MitoB – MitoBoronic Acid

MitoPerox – Mitochondria-targeted lipid peroxidation

MitoQ10 – Mitoquinone10

MitoSOX – Mitochondrial Superoxide indicator

MPC2 – Mitochondrial Pyruvate Carrier 2

mPTP – Mitochondrial permeability transition pore

mtDNA – Mitochondrial DNA

mtFAO – Mitochondrial Fatty Acid Oxidation

NAFLD – Non-alcoholic Fatty Liver Disease

NASH – Non-alcoholic Steatohepatitis

NO – Nitric Oxide

NRF1 – Nuclear Respiratory Factor 1

OMM – Outer Mitochondrial Membrane

OXPHOS – Oxidative Phosphorylation

PI3K – Phosphoinositide 3-Kinase

PPAR – Peroxisome proliferator-activated receptor

PUFAs – Polyunsaturated Fatty Acids

RAS – Renin–Angiotensin system

ROS – Reactive Oxygen Species

sdLDL – Small Dense Low-density Lipoproteins

SIRT - Sirtuin

SOD – Superoxide Dismutase

SR – Sarcoplasmic Reticulum

SS-31 – Elamipretide

STZ – Streptozotocin

TCA – Tricarboxylic Acid

Tfam – Mitochondrial Transcription Factor 1

TG – Triglycerides

Tmem135 – Transmembrane Protein 135

UCP – Uncoupling Protein

VDACs – Voltage-dependent Anion-selective Channel

WHO – World Health Organization

WT – Wild-type

1. Cardiovascular risk: definition and epidemiology

Cardiovascular diseases (CVDs) accounts for one in every three deaths and is the leading cause of mortality worldwide. It currently accounts for 17.5 million deaths annually, and is predicted to increase to 23.6 million by 2030 [1]. It is also estimated by the World Health Organization (WHO) that high blood pressure-related premature deaths will affect 1.56 billion people in 2025 [2]. Several diseases are included in the CVDs umbrella, including coronary artery disease, atherosclerosis, thrombosis, strokes, rheumatic heart disease, cardiomyopathy, ischemic heart disease (IHD) and heart failure, which collectively affect around 128 million people worldwide [3] (Figure 1). Surprisingly, the majority of CVDs cases were reported in low- and middle-income countries [4].

CVDs etiology can be considered to be a mechanistic process that is triggered by cardiovascular risk factors that results in organ damage, organ failure and finally, death [5,6]. A risk factor can thus be linked to a particular physiological mechanism, which if intervened, can halt this pathophysiological process [7]. Therefore, identifying the risk factors that predispose an individual to CVDs through large-scale epidemiological studies is a critical first step [5,8,9]. Risk factors can be divided in two groups: modifiable risk factors, which comprise of individual behaviors that predispose to CVDs, and non-modifiable risks, which cannot be altered (e.g., age, gender, genetics) [10] (Figure 1). Epidemiological studies have identified more than 60 risk factors which correlate with CVDs prevalence including high blood pressure, smoking, abdominal obesity, abnormal circulating lipids, insulin resistance/diabetes mellitus, stress, poor diet, and lack of regular physical activity, among other behavioral and environmental factors [11]. This huge number of variables makes it difficult to determine which risks are causal (e.g., high blood pressure) and which are associative (e.g., social environment).

In 70% of cases, there is a clustering of individual risk factors which synergistically increase overall CVDs risk from 4-fold with only 1 risk factor to 60-fold with 5 associated risk factors [7]. Thus, 80% of IHD deaths and 70% of stroke-related deaths are associated with small increases in different CVDs risk factors such as smoking, high Body Mass Index (BMI), alcohol use, poor diet, physical inactivity, high blood pressure and high serum cholesterol [12]. Interestingly, CVDs are most prevalent in individuals with modest increases among several risk factors as opposed to those with a few risk factors that are highly elevated [7].

The Framingham Heart Study was one of the first epidemiological studies focusing on CVDs risk factors in a stable group of subjects, with their first volunteers recruited in 1948. This study played a central role in establishing of the association of CVDs development with smoking, high blood pressure, elevated serum cholesterol levels, and diabetes mellitus [8].

The three main causes of mortality in industrialized countries are tobacco use, high levels of circulating cholesterol and high blood pressure, which in combination represent a high-profile risk factor for CVDs. The same trend has also been recently observed in developing countries with the rise in obesity and high levels of circulating cholesterol [7]. Age-standardized CVDs rates were found to be associated with the establishment of several risk factors including BMI, and were inversely correlated with per-capita GDP. Thus, the highest rates were found in the poorer countries of central and eastern Europe and central Asia representing a 4-fold increase over those of wealthy countries [11].

Although risk factors are usually considered as gender-independent factors, the incidence of CVDs are more frequent in women than men. Additional risk factors for women include disorders related with pregnancy, such as gestational high blood pressure and gestational diabetes, endocrine disorders in women of reproductive age such as polycystic

ovary syndrome and early menopause, increased abdominal obesity, and decreased levels of circulating high density lipoprotein (HDL) post-menopause [13,14].

Obesity represents a major cardiovascular risk and is tightly linked to other risk factors such as diabetes, metabolic syndrome, high blood pressure and dyslipidemia [15]. Increased BMI associated with other risk factors is responsible for nearly 9.7 million CVDs deaths annually [11]. Obesity rates have increased in recent decades, and in 2016 1.9 billion individuals were overweight, representing one-third of the global adult population [16,17]. Obesity represents a cluster of CVDs risk factors including high blood pressure, dyslipidemia, and insulin resistance. Metabolic syndrome, which represents an amalgamation of these risk factors, affects 20% to 30% of people worldwide and represents an increased CVDs risk of 70-90% [14,18].

In relation to obesity, dietary habits have a key role in individual predisposition for metabolic diseases as well as CVDs. Increased risk of CVDs was associated with specific diet profiles, including low intake of fruit and vegetables, high intake levels of trans fats, salt, processed meat, sugar, and other processed carbohydrates [11]. The magnitude of the diet effect in CVDs risk is probably exaggerated by the development of other risk factors, such as increases in BMI or blood glucose [18]. Other uncontrollable factors such as genetics as well as intrauterine and early life nutrition, can lead to the development of obesity and diabetes thereby increasing CVDs risk [19,20].

In a study standardized for genetic variations in alcohol metabolism, causal links between alcohol intake and development of IHD and stroke were identified [21]. Thus, the burden of CVDs is particularly high in countries where per-capita alcohol consumption is high such as Russia, Belarus and Moldavia as well as other ex-soviet countries [22].

Several other behavioral factors were correlated with the risk of CVDs including physical inactivity, smoking and sleep time. Worldwide, 2.5 million CVDs deaths were associated with physical inactivity [23]. The relationship between lack of physical activity and increased CVDs risk seems clear, although the association becomes non-linear for people with highly active lifestyles [24,25]. Physical activity has been shown to reduce the mortality rate in people with pre-existing coronary artery disease [26]. Sleep times that were either too short (< 6 hours) or too long (> 9 hours) were also associated with high blood pressure and metabolic syndrome which are two well-established CVDs risk factors [27,28].

Environmental risk factors were also observed for both short- and long-term air pollution exposure, which is related to IHD, stroke and heart failure (HF) incidence [29]. Reports estimate that globally, 2.5 million CVDs deaths are associated with the increase of particulate matter levels with major clusters of incidences in East and South Asia [23,31].

Alterations in these behavioral CVDs risk factors can result in a decrease in total CVDs risk. Cessation of smoking immediately starts to decrease the risk of CVDs, reaching the level for non-smokers within 10 years [30]. Also, normalization of elevated blood pressure and serum cholesterol levels immediately cut the risks of IHD and stroke, and if maintained for 5 years, the risks are reduced to that of the general population [11].

2. Altered metabolism in cardiovascular risk

2.1 – Insulin resistance and cardiovascular risk

As described above, hyperglycemia, inflammation and insulin resistance are risk factors for CVDs [33] (Figure 2). Insulin is a key regulator of cellular metabolism in various

tissues, but this can be modulated by both physiological states, for example feeding and fasting, and pathophysiological conditions such as Type 2 diabetes and fatty liver disease. Insulin sensitivity refers to the efficacy of insulin in exerting its metabolic effects, while its corollary, insulin resistance, refers to the degree by which these insulin actions are attenuated - for example by disruption of the intracellular insulin signaling cascade. Insulin resistance results in impaired glucose uptake, oxidation and conversion to glycogen. Insulin resistance is also linked to hypertriglyceridemia and low levels of circulating HDL. Moreover, insulin resistance exists in about 30% of subjects diagnosed with high blood pressure [34], while one particular study showed a direct relation to atherosclerosis [35]. Many studies have shown that insulin resistance is a good predictor of CVDs [36,37]. A follow-up study of non-diabetic young subjects revealed that about 42% of myocardial infarctions could be avoided by improving insulin sensitivity [38]. Although several studies support the perception that CVDs are correlated to insulin resistance [39–45] some contrary results have also been reported. For instance, Kozakova *et al.* concluded that insulin sensitivity associated with CVDs risk in men, while proposing that the formation of atherosclerosis and plaque were independently related with fasting plasma glucose levels in women [46]. Hyperglycemia with insulin resistance produces alterations in various cellular and metabolic functions [40,47,48] including dyslipidemia, endothelial dysfunction, high blood pressure, oxidative stress and cardiac metabolic alterations (Figure 2). Long chain fatty acid oxidation is the main pathway used for ATP (adenosine triphosphate) production in the adult myocardium; yet, the heart has the metabolic flexibility to oxidize other substrates according to their availability, including glucose, amino acids and lactate. Substrate transporters, such as CD36 (cluster of differentiation 36) for fatty acids and GLUT4 (Glucose transporter type 4) for glucose, have a high dynamic range in relation to transport rates hence the myocytes can

experience large fluctuations in glucose and fatty acid availability depending on the systemic supply of these substrates [49]. Normally about 50–70% of myocardial ATP is obtained by the oxidation of long-chain fatty acids, whereas less than 10% is derived from glycolysis [49]. During injury, the heart changes its energetic substrate preference from fatty acids to glucose. Insulin resistance impairs this metabolic flexibility and fatty acids are imported as the main fuel source. Among other things, this can result in the accumulation of lipids in the heart, which can lead to lipotoxicity [33,50]. In this context, pharmacological modulation of CD36 and GLUT4 transporter activities could be a strategy to restore glucose oxidation in the diabetic heart [49].

Moreover, insulin resistance is responsible for a decrease in endothelial nitric oxide (NO) production while at the same time it leads to increased release of pro-coagulant factors that leads to aggregation of platelets – which also promotes endothelial cell dysfunction. During insulin resistance, the mitogen-activated protein kinase (MAPK) signaling pathway remains active while the Phosphoinositide 3-kinase (PI3K) pathway is impaired, resulting in an mitogenic effect in endothelial cells that eventually leads to atherosclerosis [51,52].

2.2. Hyperglycemia in CVDs

The high CVDs risk in type 2 diabetes patients, in which CVDs incidence is 2-8 fold higher compared to normoglycemic subjects, lowers the life expectancy and accounts for the majority of fatalities globally [53,54]. One study reported that fasting blood glucose above 90 mg/dl is an independent predictor of artery atherosclerosis [55]. Impairment of glucose homeostasis, which is already present in subjects with impaired glucose tolerance

and/or impaired fasting glucose (the so-called pre-diabetic conditions) could affect the autonomic cardiac function leading to elevated CVDs risk [56].

Following the high excursions of plasma glucose levels that is characteristic of Type 2 diabetics, the hyperglycemic stress memory is retained even after control of blood glucose levels has been restored [57,58]. Hyperglycemia and glucose fluctuations activate inflammatory responses through endoplasmic reticulum stress and mitochondrial dysfunction that later results in increased ROS (reactive oxygen species) generation, which in turn causes cellular damage [59]. Hyperglycemia can also increase the expression of pro-coagulant and pro-inflammatory factors, can increase the adhesion of leukocytes to endothelial cells, can induce apoptosis and can impair the release of NO, which in turn leads to endothelial dysfunction [47,60].

The generation of advanced glycation end products (AGEs) that are non-enzymatic alteration of lipids and proteins after exposure to increased glycemic conditions is another damaging outcome of tenacious hyperglycemia [61]. Generally, AGEs accumulate in the wall of the vessel and affect the extracellular matrix (ECM) structural integrity that later leads to damage of the endothelium. This results in a decline in NO, contributing to the development of CVDs and microvascular complications such as retinopathy and nephropathy [62].

2.3. Dyslipidemia in CVDs

Impaired lipid homeostasis and storage within adipocytes secondary to factors such as insulin resistance leads to the progression of dyslipidemia. This is characterized by the lipid triad; low levels of high-density lipoprotein cholesterol (HDL-C), elevated plasma triglyceride levels, and the presence of small dense low-density lipoproteins (sdLDL), has

been documented as a noticeable risk factor for CVDs [63–65] (Figure 2). In fact, the prevalence of CVDs increases by 32% in men and 76% in women due to hypertriglyceridemia [66,67].

The accumulation of toxic lipid species (lipotoxicity), resulting from the surplus of lipids in the cardiomyocyte alters the cardiac structure and cellular signaling. Thus, lipotoxicity has been associated with several cellular signaling pathway disruptions mainly in endoplasmic reticulum stress and mitochondrial dysfunction [33]. ROS, NO, ceramide, phosphatidylinositol-3-kinase, diacylglycerol (DAG), ligands of Peroxisome Proliferator-activated Receptor (PPAR) nuclear receptors and leptin are among the mediators of these lipotoxic effects [68]. The excessive production of ROS resulting from lipotoxicity leads to DNA, protein, and membrane damage, the latter causing stress and damage within the endoplasmic reticulum. Furthermore, endoplasmic reticulum stress and oxidative stress both promote increases in intracellular Ca^{2+} [69]. Excessive, non-regulated Ca^{2+} uptake by mitochondria can result in its overload in the matrix, and to the induction of mitochondrial permeability transition pore opening. This in turn results in cell apoptosis or necroptosis and mitochondrial dysfunction, processes that are implicated in the pathogenesis of diabetic cardiomyopathy [70,71].

3. Mitochondrial roles in the cardiovascular system

Mitochondria, which are the powerhouses of cardiac cells, sustain the energetic requirements of myocardial contractile work. These organelles regulate a vast range of processes, such as ATP production, intermediary metabolism and cell death [72–75]. Mitochondria are not static entities, but instead rather dynamic units that undergo fission and fusion cycles essential for their structural integrity [76]. Also, mitochondria possess their own independent genome that encodes 13 subunits of the mitochondrial oxidative

phosphorylation (OXPHOS) complexes, multiple ribosomal and transfer RNAs, and regulatory peptides such as the recently-identified MOTS-c and humanin [77,78]. Structurally, mitochondria have two separately and functionally distinct membranes that delimitate two different mitochondrial compartments: the intermembrane space is localized between the outer mitochondrial membrane (OMM) and the inner mitochondrial membrane (IMM), while the matrix is the space within the IMM (Fig. 3). Although initially considered part of the IMM, cristae are now considered membrane-delimited independent structures which are rich in proteins responsible for ATP generation [79]. Located in the IMM is the respiratory chain where a series of redox reactions create a proton electrochemical gradient through the pumping of protons to the intermembrane space. The re-entrance of those protons to the mitochondrial matrix through ATP synthase (or complex V) drives the phosphorylation of ADP (adenosine diphosphate) to ATP [80]. In cardiomyocytes, mitochondria are densely condensed, occupying around one third of cell volume, in order to respond to the high cardiac energy demand [81,82]. In fact, almost all the ATP (>90%) required for the contraction and relaxation loop is provided by mitochondrial oxidative phosphorylation and ATP needs to be constantly synthesized from ADP and inorganic phosphate [83,84]. Fatty acids are the preferred fuel for mitochondrial ATP production in the heart, yet the tissue can utilize many other substrates including carbohydrates, amino acids and ketone bodies depending on physiological conditions and substrate availability [85–87]. Physiologically, the mechanism of ATP production in a normal heart occurs usually in the following sequence: fatty acyl-CoA and pyruvate, resulting from fatty acids and glucose metabolism, respectively, are transported across the IMM and oxidized to acetyl-CoA. Pyruvate conversion is regulated by the pyruvate dehydrogenase complex, while fatty acyl-CoA undergoes fatty acid β-oxidation (FAO), and the resulting acetyl-CoA enters the TCA (tricarboxylic acid) cycle

generating NADH and succinate that reduces the electron transport chain (ETC). The majority of the ATP generated from this process is then hydrolyzed to fuel the contraction machinery. ATP is required to dissociate actin from myosin [88], whereas the remaining amount is mostly used by transmembrane ion pumps [86]. As such, mitochondria play a key role in the maintenance of cytosolic Ca^{2+} concentration and modulation of muscle contraction. Briefly, during contraction-relaxation cycles electric stimulation evokes the influx of Ca^{2+} from the extracellular milieu to the cytosol and later from the sarcoplasmic reticulum (SR). The increased concentration of cytosolic Ca^{2+} induces the contraction mechanism (through the binding of Ca^{2+} to troponin C) that is then followed by relaxation when Ca^{2+} is pumped out of the cell or transported back to the SR. During this process, mitochondria not only act as local buffers, but also the mitochondrial uptake of Ca^{2+} alters the activity of several mitochondrial enzymes resulting in the increase of respiratory rate and ATP production [89] (Figure 3). While the OMM is very permeable to Ca^{2+}, mostly because of the presence of voltage-dependent anion-selective channel proteins (VDACs), the entrance of calcium into the matrix occurs essentially through the mitochondrial Ca^{2+} uniporter (MCU), driven by the mitochondrial membrane potential [90]. Despite the low affinity of MCU to Ca^{2+}, the proximity of mitochondria to the SR and the formation of SR-mitochondria membrane contact sites leads to the transfer of Ca^{2+} directly from SR stores to the mitochondrial matrix [91–93], being then counteracted by the mitochondrial Na^{+}/Ca^{2+} and H^{+}/Ca^{2+} exchangers [94]. Given the importance of mitochondria to the cardiac activity and blood pumping, any decline of mitochondrial function is associated with impaired heart function [95]. The mitochondrial abnormalities may be associated with a reduced generation of ATP, increased production of ROS or even mitochondrial-driven cardiomyocyte death.

4. Role of mitochondrial alterations in cardiovascular risk

This section will give some examples of specific conditions/pathologies in which an association with altered cardiac mitochondrial function has been described.

4.1 – Obesity

Excessive fat accumulation in adipoctyes as well as in ectopic sites such as hepatocytes, pancreas and myocytes, increases the risk of developing a number of patho-physiological conditions, including CVDs. In an editorial comment for the Journal of the American College of Cardiology [96], Dale Abel briefly reviewed possible mechanisms that may contribute to cardiac alterations observed in obesity. The comment drew attention to the changes in cardiomyocyte metabolism, which include alterations in mitochondrial function. His comment was based on a study developed by Niemann and co-workers, also published in the same journal issue [97]. In this study of human adult right atrial cardiomyocytes, it was demonstrated that obesity disturbed both mitochondrial biogenesis and function, decreasing the mRNA levels for NRF1 (nuclear respiratory factor 1) and Tfam (mitochondrial transcription factor 1), two important transcription factors involved in mitochondrial biogenesis, and reducing the levels of mRNA and protein for ND6 (subunit of mitochondrial respiratory complex I, encoded in mitochondrial DNA) and NDUFB8 (another subunit of mitochondrial respiratory complex I, encoded in nuclear DNA). Furthermore, a decrease in the activity of mitochondrial respiratory complex I was also observed in atrial cardiac cells from obese patients. Those changes suggest that in obesity, mitochondrial function in cardiac cells appear to be compromised, which may be associated with an increased risk of HF. In fact, higher markers of oxidative stress were also observed in cardiac cells isolated from young obese as well as a 30% decrease in telomerase length - a sensitive indicator of

cumulative oxidative stress. Furthermore, higher expression of the pro-apoptotic proteins Bax and BCL-xS, the presence of cytochrome c in the cytosolic fraction of cardiac cells, as well as an increase amount of cleaved caspase 9, suggest an activation of the mitochondrial dependent apoptotic pathway in cardiac cells of obese individuals.

Mitochondria that were abnormally large size and devoid of cristae were also observed in cardiac tissues harvested from obese mice [98]. The authors also observed higher levels of 4-hydroxy-2-nonenal (4HNE), a marker of lipid peroxidation but low levels of mitofusin (MFN) 2, an outer mitochondrial membrane GTPase required for the process of mitochondrial fusion. This suggests a disruption in mitochondrial quality control mechanisms which play a fundamental role in the maintenance of cardiac function [99]. In a rodent model, obesity led to a decreased expression of the mitochondrial SIRT3, which at least partly resulted in cardiac remodeling and dysfunction [100]. In other tissues, decreased SIRT3 expression and activity resulting from a high fat diet also led to increased acetylation of mitochondrial complex I, thereby reducing its activity [101]. Whether this same process occurs in the heart is not yet established.

Mitochondrial impairments were also observed in neonatal overfed rats, with decreased mitochondrial coupling efficiency and increased oxidative stress being observed [102]. These findings suggested that correct nutrition in critical periods of development are important in reducing the risk of cardiovascular problems.

Maternal obesity is associated with increased risk of cardiovascular dysfunction in the offspring. Ferey *et al*. demonstrated a transgenerational cardiac mitochondrial dysfunction [103], which was independent from maternal mitochondria inheritance. The authors observed that the offspring of obese mothers showed cardiac mitochondrial abnormalities, developing left ventricular hypertrophy. However, those effects observed

in the male F1 generation were also transmitted to their descendants, suggesting epigenetic nuclear alteration in germ cells of obese mothers.

In conclusion, changes in cardiac mitochondrial function and dynamics are observed in obese individuals. Disruption in mitochondrial quality control mechanisms in obesity can increase the risk of cardiac cell death and consequently HF, especially under stressful conditions.

4.2. Aging

Aging is a natural process among living organisms, and is characterized by physiological changes and cellular death (phenomenon of growth, decline and death). López-Otín *et al*. described in 2013 nine hallmarks of aging: genomic instability, telomere attrition, epigenetic alterations, loss of proteostasis, deregulated nutrient-sensing, mitochondrial dysfunction, cellular senescence, stem cell exhaustion, and altered intercellular communication [104]. Mitochondrial function has been related with aging-related consequences in the past decade [105–108], with a decrease in mitochondrial function accounting for alterations of different signaling and metabolic pathways. According to the free radical theory of aging there is an excessive production of ROS due to progressive mitochondrial dysfunction [109,110] leading to an increase of oxidative damage, although more recent data casted doubt of this model of aging, showing that physiological levels of ROS may not be harmful to mitochondria [105].

Since ROS production also occurs in mitochondria (especially at the mitochondrial respiratory chain complexes I and III), this organelle is more susceptible to oxidative damage with advancing age. Post-translational modifications, such as acetylation of mitochondrial proteins by mitochondrial sirtuins (SIRT3, SIRT4 and SIRT5), has been shown to be involved in the pathogenesis of cardiac diseases such as myocardial

infarction (ischemia-reperfusion (IR)) and HF [111,112]. SIRT3 is the main NAD^+-dependent mitochondrial deacetylase and regulates mitochondrial bioenergetics and metabolism, contributing to the prevention of the redox stress and cell aging [111]. SIRT3 is also responsible for the regulation of the mPTP (mitochondrial permeability transition pore). mPTP opening is related with different pathologies and with the release of proapoptotic factors. SIRT 3 can inhibit mPTP opening, via deacetylation of cyclophilin D– a key component of mPTP. This cascade of events will eventually reduce oxidative stress and slow down cardiac aging [113].SIRT3 KO mice showed decreased expression of angiogenic growth factors, endothelial dysfunction and coronary microvascular dysfunction post-myocardial ischemia (MI), leading to impaired cardiac recovery. On the other hand, overexpression of SIRT3 protected heart from MI [114]. In addition to SIRT3, the mitochondrial $p66^{Shc}$ adaptor protein has been linked to aging and CVDs [115,116]. In a knockout mouse model, $p66^{Shc}$ genetic deletion induced a decrease of ROS levels and at the same time resulted in a prolonged lifespan [117]. With advanced age, endothelial dysfunction of the basilar artery is reduced in $p66^{Shc-/-}$ mice comparing with age-matched WT mice due to a lower ROS production, thus reducing a risk factor for stroke [118]. Also, hypertensive Wistar Kyoto rats presented a higher activation of $p66^{Shc}$ in isolated aortic endothelial cells, compared to normotensive ones [119]. Likewise, knockout mice have smaller strokes after IR brain injury [120]. Alterations of transmembrane protein 135 (Tmem135) gene expression, a regulator of mitochondrial dynamics [121], have also been found to be related with heart abnormalities [122]. Mitochondrial size in cardiomyocytes is decreased in transgenic mice overexpressing Tmem135 compared to wild-type (WT) mice. Moreover, transgenic hearts for Tmem135 shows similar gene expression profiles and pathologies to aged hearts such as hypertrophy and collagen accumulation, indicating fibrosis [123]. It is known that some

cardiomyopathies may be attributed to the accumulation of mitochondrial DNA (mtDNA) damage [124,125], which can lead to defects in OXPHOS enzyme genes and decreased apoptotic threshold [126]. In an early study, Matthews *et al*. correlated the 3243 A to G mtDNA mutation to an increase in cardiomyopathy incidence [127]. More recently, Tranah *et al*. (2018) quantified the 3243 A to G mtDNA mutation in an aged population, showing that some age-related diseases can be attributed to the accumulation of mtDNA damage [128].

Although aging is a natural process, its progression is linked to a higher incidence of CVDs. Cardiac mitochondrial function deteriorates during the aging process, which limits not only the capacity of the heart to withstand second stresses, but also reduces cardiac contractile performance not only because of lack of necessary ATP, but also because of imperfect regulation of cytosolic calcium and redox balance.

4.3 – Diabetes

Although the increased risk of HF during diabetes is multifactorial, changes in mitochondrial function appear to have determinant role [129,130]. In the diabetic heart, a metabolic shift towards more active FAO is observed, which is associated with a reduction in cardiac efficiency [130–133]. Changes in acetylation/deacetylation of mitochondrial proteins have been considered key regulators in mitochondrial metabolism shift in diabetic heart [111]. For example, hyperacetylation of mitochondrial pyruvate carrier 2 (MPC2) is observed in a transgenic mouse model for type I diabetes, decreasing the pyruvate transport in heart mitochondria and contributing to the metabolic changes [134], since it decreases the flux of the Krebs cycle and feeding of electrons to the mitochondrial respiratory chain.

Cardiac contractile dysfunction and an impaired mitochondrial function was observed in the heart of SIRT3 knockout mice, when compared to their wild type counterparts. In line with the absence of SIRT3, hyperacetylation of several proteins of mitochondrial energy catabolic pathways have been identified as a major cause for the observed cardiac dysfunction [135]. The role of SIRT3 for the development of diabetes-associated cardiomyopathies appears to be determinant. However, how SIRT3 activity is decreased during hyperglycemia or insulin resistance is not entirely clear. A reduction in the expression and activity of SIRT3 was observed in fetal endothelial colony-forming cells (ECFCs) and human umbilical vein endothelial cells (HUVECs) isolated from cord and cord blood of gestational diabetes pregnancies, suggesting a possible mechanism for the long-term cardiovascular complications observed in the offspring of gestational diabetes pregnancies [136]. Thus, hyperacetylation of enzymes involved in mitochondrial metabolism appears to contribute for diabetes-associated cardiac dysfunction.

$p66^{Shc}$ is also linked to cardiovascular dysfunction and oxidative stress markers that occur in diabetes, and can be a powerful therapeutic target to vascular complications that come with this pathology [137,138].

Changes in lipid profile, with an increased production of toxic lipids species, reduction in polyunsaturated fatty acids (PUFAs) and downregulation of several phospholipid species were observed in cardiac tissue of C57BL/6 male mice injected intraperitoneally with a single dose of 150 mg/kg body weight of streptozotocin (STZ) [139]. An increased expression of gene involved in fatty-acid degradation and increased peroxisomal beta oxidation as well as abnormalities in mitochondrial structure and decreased ATP levels were also observed in cardiac tissue of animals treated with STZ. The authors suggested that those abnormalities in mitochondrial structure and decreased ATP levels could be related with the changes in phospholipid profile. Interesting, the authors also found an

increase in mRNA levels of mitochondrial uncoupling protein 3 (UCP3), which was associated with an increased in mitochondria uncoupling and decreased of ATP levels. However, another study demonstrated a down-regulation in mitochondrial UCP3 in myocardial tissue from animal models of insulin resistance and type 2 diabetes, impairing mitochondrial fatty acid oxidation (mtFAO) and contractile recovery after an IR episode [140]. These contradictory results may be related to the different animal models used in the two studies, indicating that different mechanisms may be involved in diabetes-associated cardiomyopathies, depending on the individual genetic profile and diabetes-induced stimulus.

Notably, a decreased tolerance to the induction of the calcium-induced mitochondrial permeability transition has also been observed for hearts of diabetic animals, which can promote the loss of cardiomyocytes by apoptosis or necroptosis [141–143]. Augmented induction of the mitochondrial permeability transition pore can disrupt the role mitochondria have in calcium handling and ROS generation in the myocardium, contributing to contractile disruption [144].

In OVE26 mice, cardiac dysfunction was observed as early as 3 months of age. This mouse model is built around a 5-fold increase in calmodulin expression in beta cells due to a calmodulin minigene driven by the rat insulin promoter which leads to beta cells apoptosis and consequent hyperglycaemia by 2-3 weeks of age [145–147]. In this model a large decrease in mitochondrial respiration when stimulated with non-FA substrates was observed, which is probably a result of mitochondrial dysfunction resulted from the accumulation of incomplete FA oxidation molecules [148,149].

By using the AKITA Ins2+/- mouse, compromised cardiac mitochondrial function without alterations in cardiac efficiency or in insulin resistance was also previously observed [150,151]. This model is characterized by a mutation in insulin 2 gene which

impairs protein folding resulting in a progressive loss of beta cells similarly to what occurs in T1D [152,153]. Cardiac lipid metabolism as well as lipid phenotype is affected in this model. An increase in fatty acyl – CoA, ceramides, DAG and TAG was observed in 3 months AKITA mice, a time at which lipid droplets could be observed in the heart. which are more pronounced at 6 months of age [154]. Furthermore, increased mitochondrial FA oxidation was described with concomitant increase in mitochondrial FA oxidation proteins, ATGL, PDK4, CD36 and FATP expression [150,151,154,155].

In conclusion, mitochondrial metabolism appears to have a determinant role for the development of cardiomyopathies induced by diabetes. The shift towards FAO in detriment to glycolysis results in accumulation of toxic lipids intermediates and alteration in cellular lipid profile, having a negative effect on mitochondrial function. Since mitochondria are the main producer of ATP in cardiac cells, any damage in this organelle compromises viability and function of those cells and consequent performance of the heart.

4.4. Non-alcoholic fatty liver disease (NAFLD)

The abnormal accumulation of lipids, mainly triglyceride, in the liver in the absence of (or low) alcohol ingestion is known as NAFLD [156]. NAFLD can evolve to non-alcoholic steatohepatitis (NASH), fibrosis, cirrhosis and liver failure, and hepatocellular carcinoma [157]. NAFLD frequency is growing worldwide and is related to the increase of cardiovascular risk and other metabolic syndromes such as insulin resistance and obesity [158–160]. Cardiovascular disease is one of the most common cause of mortality in NAFLD [161] and some authors concluded that cardiac dysfunction is also associated with visceral fat accumulation – itself frequently associated with NAFLD [162]. Mitochondria are present in a large number in hepatocytes ranging from 500 to 4000 per

hepatocyte [163] and mitochondrial dysfunction has been related to the progression of NAFLD (Figure 4), since those organelles lose the ability to oxidize fatty acids, increasing their accumulation in the hepatocyte [163–165]. Mitochondria play a key role in the maintenance of fat homeostasis, but also in the maintenance of ROS levels that can trigger lipid peroxidation, cytokine overproduction and apoptosis if ROS production is uncontrolled [166]. Hepatocyte free fatty acids (FFA) undergo mtFAO regulated by carnitine palmitoyltransferases (CPT) I and II producing ROS (Figure 5), so the increase of FFA in NAFLD will lead to oxidative stress and inflammation [167]. As described previously, the heart obtains most of its chemical energy from FAO, and a state of cardiac lipotoxicity can be reached when there is increased fat deposition in cardiomyocytes as well as in the epicardial adipose tissue surrounding the heart [168]. A 2016 study showed that increased epicardial adipose tissue volume and NAFLD are associated with the presence of the metabolic syndrome [169], while a more recent study showed that epicardial adipose tissue is strongly associated with NAFLD and other cardiovascular risk factors [170]. To our best knowledge, there are no reports that have presented evidence linking alterations in epicardial adipose tissue mitochondrial activity with altered contractile performance of the myocardium.

Mitochondrial dysfunction has been related to several cardiac abnormalities in models of high-fat diet (HFD). Recently, Nie H. *et al* observed a reduction in the activity of cardiac mitochondrial complex I with a reduction of ATP production and oxygen consumption in obese-mice. Along with these features, mice presented cardiac hypertrophy and severe cardiac structural disorders [171]. Those results were supported by other authors using other rodent models, together with the observation of morphologic changes in mitochondria and a reduction in mtDNA copy number in HFD group, as well as reduced mitochondrial fusion genes (MFN1, MFN2 and OPA1) and enhanced mitochondrial

fission genes (DRP1 and FIS1) [172]. Cardiac mitochondrial lipid profile was also found to be altered in obese when compared to their non-obese counterparts. Cardiac mitochondrial triglycerides (TG), independently associated with myocardial fibrosis, were increased in HFD-fed rats with myocardial infarction, as opposed to overall cardiac mitochondrial cardiolipins (CLs) whose levels were reduced [173]. Cardiac ceramides (CER) are related to cardiovascular events [174], and were significantly increased in mitochondria of heart from rats HFD with MI [173]. Murray *et al.* showed that rats with failing hearts presented higher levels of UCP3 and high circulating FFA concentrations [175]. The cardiac tissue overexpresses UCP2 and 3 as a mechanism against lipotoxicity and excessive ROS production [176], which can both act as a potential mechanism and therapeutic target for NAFLD [177]. However, a study in UCP2 knock-out mice exposed to an obesogenic stimulus did not reveal any differences on severity of NAFLD comparing with mice expressing UCP2 [178].

The published data so far indicates that NAFLD is associated with cardiovascular alterations, which involve increased accumulation of fat in cardiomyocytes while at the same time mitochondrial activity is decreased. Among other things, contractile perturbances can be a consequence of this disarranged cardiac metabolic state.

4.5 – Kidney diseases

The dynamic interplay between heart and kidney dysfunction is described under the umbrella term named cardio-renal syndrome. Although not a new concept [179], cardio-renal syndrome become more recognized after the consensus conference by the Acute Dialysis Quality Initiative (ADQI), where a definition and a classification in 5 types were defined [180–182]. The consensus definition for cardio-renal syndromes was termed as: “disorders of the heart and kidneys whereby acute or chronic dysfunction in one organ

may induce acute or chronic dysfunction of the other" [180]. Cardio-renal syndromes were also classified in five types: *Acute cardio-renal syndrome* (type 1), where an acute HF induces an acute kidney injury; *Chronic cardio-renal Syndrome* (type 2), where chronic cardiac dysfunction induces progressive kidney injury; *Acute reno-cardiac syndrome* (type 3), where an acute kidney dysfunction induces heart injuries; *Chronic reno-cardiac syndrome* (type 4), where chronic kidney dysfunction induces progressive HF; and *Secondary cardio-renal syndromes* (type 5), where systemic disorders, such sepsis, infections, drugs or toxins, lupus, diabetes or other chronic inflammatory conditions, induces simultaneous injuries of the heart and kidneys. Both the kidney and heart are high energy-demanding organs, hence mitochondria play in both organs essential roles. Bigelman and co-workers [183] demonstrated that chronic kidney disease (CKD) induced changes in the structure of cardiac mitochondria, including increased volume indicative of mitochondrial swelling (Figure 5). Furthermore, increased cytochrome c leakage to the cytosol and cleavage of PARP-1 suggested mitochondrial dependent apoptotic pathway activation. Changes in CL remodeling and loss of its content was observed in cardiac tissue from female domestic pigs with renal artery stenoses and consequent renovascular high blood pressure [184]. CL is a phospholipid found exclusively in mitochondrial inner membrane, with a conical structure that allows optimal assembly of the supercomplexes of the mitochondrial ETC within the mitochondrial cristae curvature [185]. Thus, changes in CL contents and remodeling disturb mitochondrial function and increase oxidative stress and apoptosis in cardiac cells leading to myocardial injury. Fragmentation of cardiac mitochondria were also found in 8-week-old male C57BL/6 mice, undergoing bilateral renal artery clamping for 30 min to induce renal IR injury [186]. Associated with mitochondrial fragmentation, apoptosis of cardiac cells and cardiac dysfunction was also observed. Increased levels of dynamin-

related protein 1 (Drp1), a protein that regulates the mitochondrial fission, was observed in the heart of C57BL/6 mice 24 hours after renal IR injury. Interesting, inhibition of Drp1 prevented mitochondrial fragmentation, cardiac cells apoptosis and cardiac dysfunction [186], suggesting that inhibition of cardiac mitochondrial fission could in theory have a therapeutic effect during acute kidney injuries.

As a conclusion, changes in mitochondrial dynamics and function are observed in cardiac tissue after acute or chronic kidney injury and may be the bridge in the cardio-renal syndrome. Thus, protecting mitochondrial function may be a good strategy to prevent cardiac injuries induced by kidney dysfunction.

5. Improving cardiac mitochondrial function to decrease cardiovascular risk

5.1. Physical activity

Regular physical activity provides irrefutable benefits for human health and it has a well-established relationship with cardiorespiratory fitness and protection (Figure 6) [187–189]. In adults, recommendations from the WHO indicate that a minimum of 150 min of moderate-to-vigorous physical activity should be performed through the week, while for children and adolescents this amount should be significantly greater (> 60 min per day) [190,191]. In a large population study (> 55,000 adult man), around 10 min running per day at a slow speed was already associated with reduced risk of death and CVDs [192]. However, the challenging modern lifestyle progressively reduced the time dedicated to physical activity and the rising of physical inactivity has been identified as the fourth leading risk factor for global mortality [193,194]. IR insults are particularly damaging to the myocardium [195–197]. Oxygen deprivation, as a result of obstructed coronary artery, rapidly unbalance the bioenergetics supplies. However, the posterior reoxygenation by

reperfusion can be even more damaging because of a panoply of pathological mechanisms, especially the dramatically increase in ROS production [196]. The strategy of preconditioning to protect IR hearts has been extensively studied, and was first noted when brief and repetitive non-injurious ischemic episodes, before a subsequent long last ischemia, decreased heart injury caused by IR [198]. Similarly, cardiac preconditioning by exercise training (considered a more intense stimulus than physical activity) protects the heart against IR. During resistance exercise there is a drop in the blood flow to the tissues that may cause temporary ischemia. These bouts of non-deleterious IR episodes may exert protective effects on the vasculature. This strategy has been successfully applied in rodent models [199–202]. In humans, both younger (25 ± 2 years) [203] and older (> 57 years) [204] individuals following lifelong exercise trainings have more tolerance against endothelial IR compared with sedentary participants.

During physical activity or exercise the cardiovascular system needs to adapt in order to pump adequate amount of blood to exercising and non-exercising tissues. Thus, mitochondria are required to produce a larger amount of ATP to fuel the increased workload. A number of studies have focused on identifying the mitochondrial parameters that are remodeled by exercise training, even though the underlying mechanisms are still only partially understood [205–207]. Mitochondria isolated from exercised animals are less sensitive to apoptotic stimuli [208,209] and some authors observed improvements in cardiomyocyte Ca^{2+} cycling (and hence contractile function) [210] as well as decreased susceptibility to Ca^{2+}-induced mPTP opening due to an increased capacity to accumulate Ca^{2+} [211]. Moreover, it is well known that during heart disease energy metabolism switches from FAO to glycolysis. Kavazis *et al*. [207] observed that following repeated bouts of endurance exercise, several proteins involved in FAO were increased in rat cardiac mitochondria. Regarding antioxidant defenses, there is a lack of consensus on

their role in conferring protection during exercise [212]. Exercise training was found by many to increase mitochondrial SOD (superoxide dismutase) 2 activity [208,209,213], whereas inconsistent results were observed regarding cytosolic SOD1, glutathione peroxidase and catalase activity, probably because of the diversity of training protocols and different methodologies (enzyme activity measured from heart homogenates *vs* isolated mitochondria) [209,212,214]. The heat shock protein (Hsp) system, another cell defense mechanism against oxidative stress, was also involved in the exercise-related beneficial effects. In particular, Hsp70, associated with myocardial protection [215], was shown to be increased in rats after a 8-weeks training, mitigating the age-dependent decline of Hsp70 abundance [216]. In another 8-weeks aerobic exercise study, rats posteriorly subjected to myocardial infarction-induced HF presented signs of improved cardiac function (restored mitochondrial oxygen consumption, increased Ca^{2+}-induced mPTP and reduced H_2O_2 release) and improved cardiac protein quality control [205]. Also, moderate intensity exercise stimulates the synthesis of NO, an important regulator of vascular tone and blood flow [217], by increasing and activating the endothelial nitric oxide synthase (eNOS) [218]. NO regulates PGC-1α expression via the generation of cyclic GMP (cGMP) and induces the expression of several members of the mitochondrial ROS detoxification system [219,220]. In addition, PGC-1α-dependent mitochondrial biogenesis also seems to be regulated by musclin, an exercise-responsive myokine with homology to natriuretic peptides [221]. Importantly, most of the studies are based on the effects of regular physical activity on the cardiovascular system. Acute episodes of physical activity have been considered to increase the incidence of myocardial infarction, especially in sedentary individuals [222]. Overall, evidence suggests that both short-term (few days) and long-term (weeks to months) exercise improve myocardial tolerance to IR [223].

5.2 - Pharmacological interventions

It is unquestionable that the lives of millions of people in the world have been improved by the advances in primary and secondary prevention of CVDs [224]. Although there are some controversial results, a number of therapeutic approaches have been studied and validated, including lifestyle interventions (diet and physical exercise) and medications that target oxidative stress mechanisms, inflammation, cardiac hypertrophy, apoptosis and fibrosis (Figure 6) [225–227]. Molecules that can target mitochondria and decrease mitochondrial dysfunction have been developed in recent years [228]. Interestingly, the objective of many groups is to discover novel therapeutic agents that target mitochondrial function and excessive ROS production associated with the development of atherosclerosis, IR injury, diabetes mellitus, high blood pressure and HF [229].

Accordingly, several studies in both animal and human have revealed that co-enzyme Q_{10} (CoQ_{10}) present in the inner membrane of mitochondria - thus vital for ATP production - shows anti-thrombotic and antioxidant properties, improvement of high blood pressure and hyperglycemia-induced injury [230]. The study performed in hypertensive rat models have shown that CoQ_{10} supplementation improved endothelial function and cardiac hypertrophy [231]. Moreover, administration of CoQ_{10} in humans was shown to relieve the muscle pain derived from statin-caused rhabdomyolysis [232], although it is still not clear whether CVDs would all benefit from CoQ_{10} supplementation, although it has been proposed to be an adequate treatment option against mitochondrial dysfunction during high blood pressure and HF in humans [233].

Additionally, administration of antioxidant molecules linked to lipophilic molecules which selectively target the mitochondria have served as another alternative new approach [234,235]. For instance, $MitoQ_{10}$ ($Mitoquinone_{10}$) was tested in hypertensive

rat models and showed improvements on endothelial NO bioavailability and blood pressure [231]. Moreover, $MitoQ_{10}$ treatment has shown beneficial against high blood pressure [236], cardiac hypertrophy [231] and IR injury [235], although over-dosage can potentially disrupt the function of mitochondria [237]. In addition, a range of Mito-compound probes including MitoB (MitoBoronic Acid) [238], MitoSOX (mitochondrial superoxide indicator) [239] and MitoPerox (mitochondria-targeted lipid peroxidation) probe [240] have been developed in this series. MitoSOX and synthetic SOD molecules (EUK-8 and EUK-134) have shown mitochondrial antioxidant actions against IR injury [241,242]. In a different strategy, the β-blocker carvedilol has shown beneficial antioxidant and anti-apoptotic properties in HF patients [243]. Interestingly, we and others have already shown that carvedilol prevents cardiac mitochondrial oxidative damage in different model systems [244–250], in part by inhibiting the mitochondrial permeability transition pore.

Importantly, angiotensin-converting-enzyme (ACE) inhibitors and angiotensin receptor-II blockers that targets the activation of Renin–Angiotensin system (RAS) have also shown beneficial effect against mitochondrial dysfunction [251]. For instance, dogs treated with captopril showed increased cardiac mitochondrial biogenesis, which can be effective to inhibit mitochondrial disruption upon different of internal and external cellular stresses [252,253].

Statins are among the drugs which show a significant pleiotropic effect besides their known activity of inhibiting the synthesis of endogenous cholesterol [254,255]. Statins have been shown to act on mitochondria in different tissues to decrease oxidative stress [256]. Moreover, Parihar *et al.* showed in rats that the activity of mitochondrial NO synthase, cytochrome c release and lipid peroxidation were reduced by atorvastatin and simvastatin [257].

Antidiabetic drugs are among the alternative therapeutic agents involved in CVDs treatment by improving mitochondrial function. For instance, metformin which is the primary therapeutic option for newly-diagnosed type 2 diabetes mellitus, has displayed an overall sparing effect on the cardiovascular system [258]. Specifically, studies have indicated that metformin plays a vital role in reducing mitochondrial ROS production, enhancing antioxidant enzymes activity and decreasing inflammation implicated in IR injury [259]. Furthermore, thiazolidenediones are another class of antidiabetic drugs that activate PPARγ, thereby improving lipid storage in adipocytes and diminishing ectopic lipid pools. These have been shown to inhibit atherosclerosis development in animal models [260,261].

Alternatively, the novel therapeutic drug, Elamipretide (SS-31), is a water-soluble tetrapeptide that boosts energy production in mitochondria. It selectively binds to CL, conserves the mitochondrial cristae structure and enhances OXPHOS function [262]. Remarkably, in advanced HF dog models, SS-31 enhanced the enlargement and function of the left ventricle, decreased the formation of ROS, and resulted in improved plasma natriuretic peptides and inflammation biomarkers [263]. A randomized trial study in humans with HF have shown similar results [264]. In rats, SS-31 improved oxidative stress and delayed cardiac remodeling and post myocardial infarction inflammation [265].

In conclusion, a number of therapeutic options including the ones listed above have been tested for protecting mitochondrial function in individuals which have one or more risk factors, and that show early signs of CVDs.

6. Conclusions and Future directions

Because the myocardium is very dependent on mitochondrial function, not exclusively due to the role of those organelles in energy production, it is not surprising that CVD have a strong mitochondrial component. We exemplified here some risk factors (obesity,

aging, diabetes, kidney disease) that are related with decreased cardiac mitochondrial function. Regardless of the mechanisms involved, the end result is a loss of mitochondrial ability to produce ATP, to regulate calcium and other ion fluxes in the cell, to control the generation of ROS (often leading to increased ROS generation which is not counteracted by an effective antioxidant network), and to properly control cell death. Loss of contractile activity can ensue because of energy supply failure leading to pump failure. Preventing this to occur is achieved through control of modifiable risk factors, which commonly involve moving towards healthy lifestyles. The present review presents physical activity and mitochondria-directed strategies as two possible interventions that could not only manage risk factors but act directly on the mediator of tissue dysfunction. Still, there are many open challenges, including understanding how cardiac mitochondria respond to one of multiple risk factors, how to measure cardiac mitochondrial function in a non-invasive manner in order to predict disease staging or therapeutic intervention success, and the successful development and entry in the market of novel single or mixed interventions, which can act in multiple levels, not only lowering modifiable risk factors, but also directly acting to decrease mitochondrial disruption, especially resulting from increased ROS production. In the near future, it would also be interesting to understand the role of mitochondrial dynamics and mitophagy in the homeostasis of the cardiovascular system and cardiomyocyte quality control and repair, as well as the interface between metabolic dysfunction and diverse signaling pathways implicated in nuclear transcription regulation and mitochondrial biogenesis. Furthermore, non-invasive measurement of "live" cardiac mitochondrial metabolism could be used to anticipate the progression of CVDs provided that early and sensitive markers of mitochondrial dysfunction are identified.

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9. Legends for figures

Figure 1. Schematic diagram of the major CVD risk factors with in which an established relation with disease development has been determined in multiple studies.

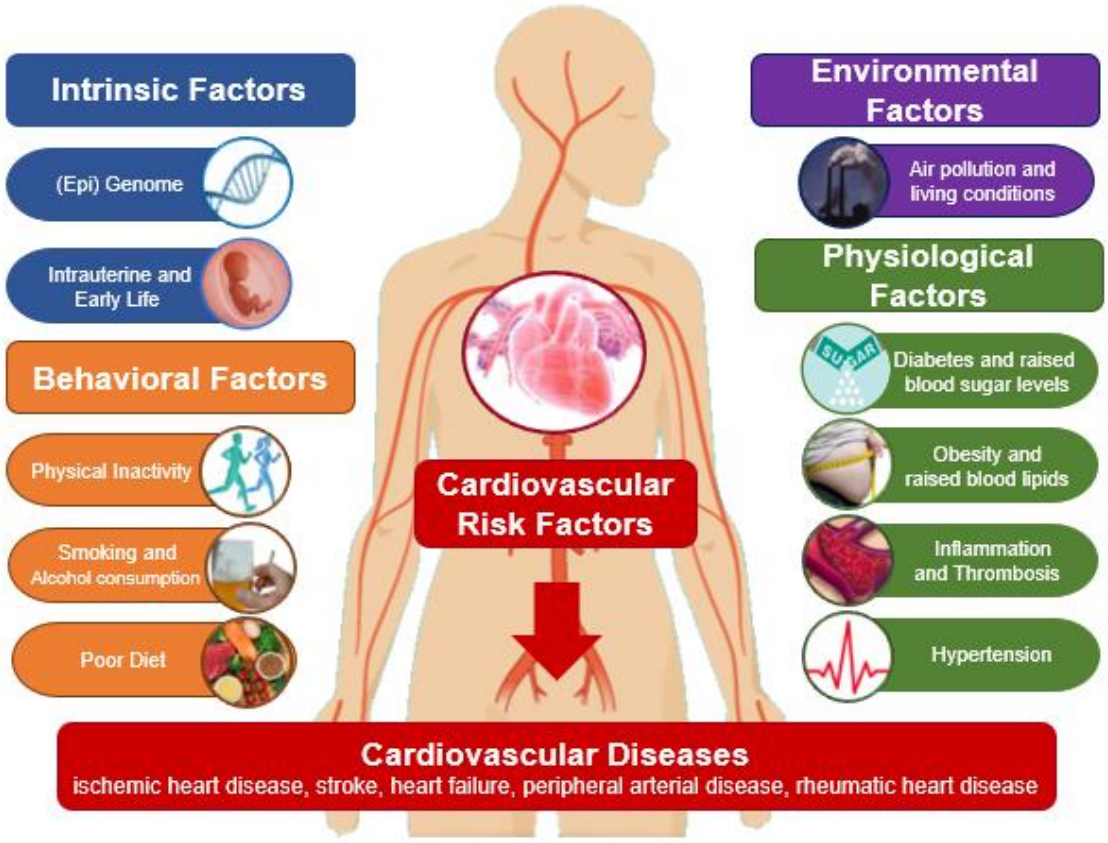
Figure 2. The contribution of altered metabolism in cardiovascular risk

Figure 3. Cardiac mitochondria are essential to maintain the energy supply required by cardiomyocytes, especially for the contraction/relaxation process. In the inner mitochondrial membrane, the four ETC complexes create a proton gradient that is used to powers ATP synthase. ATP and Ca^{2+} are both required for the contraction process. Most of ATP is consumed by ions pumps (including SERCA and Ca^{2+} ATPase) and contractile myofilaments. The increase of Ca^{2+} on the cytosol activates contraction and its release back to sarcoplasmic reticulum (SR) or the extracellular milieu leads to relaxation. Ca^{2+} enters mitochondria through VDACs and mitochondrial Ca^{2+} uniporter (MCU) complex and, when inside, it regulates ATP production and mitochondrial homeostasis. ANT, adenine nucleotide translocase; OXPHOS, oxidative phosphorylation.

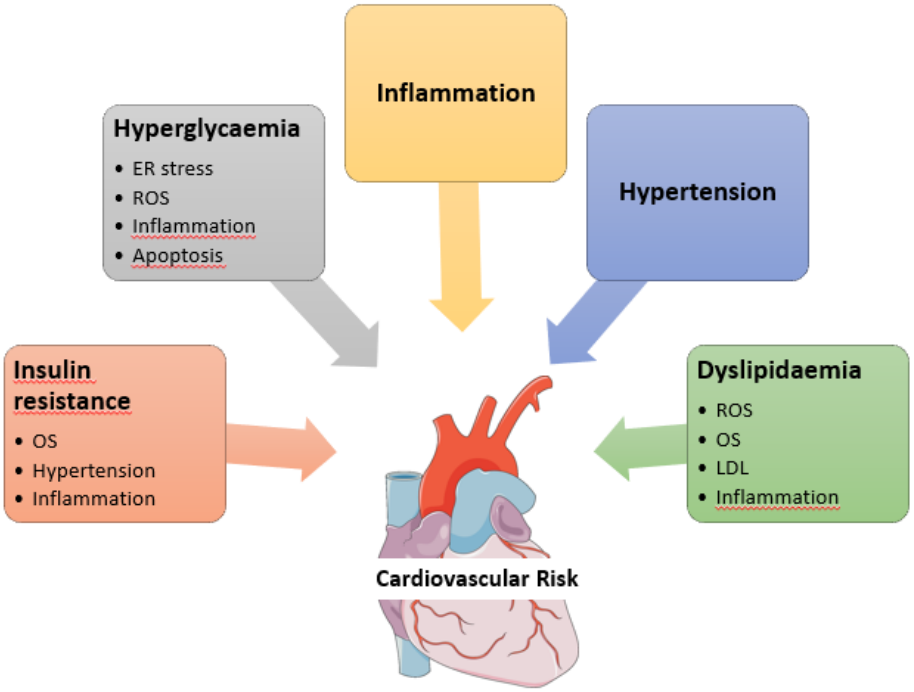
Figure 4. NAFLD leads to the increase of fat deposit accumulation in the hepatocytes, leading to a decreased ability of hepatic mitochondria to oxidize fatty acids. Often associated with dyslipidemia, one consequence of NAFLD is increased oxidative stress in other tissues, including the heart. Obesity, one risk factor for NAFLD, is often accompanied by increased lipid accumulation in the cardiac tissue and surrounding epicardial adipose tissue.

Figure 5. Changes in cardiac mitochondria induced by obesity, aging, diabetes, NAFLD and KD and risk of cardiovascular diseases. Several conditions such obesity, aging, diabetes, NAFLD and KD disturb the system and induces changes in cardiac mitochondrial function. The heart is a high-demand energy organ and relies, majority, on mitochondrial function. Damages in cardiac mitochondrial function increases the cardiovascular risk and may lead to HF.

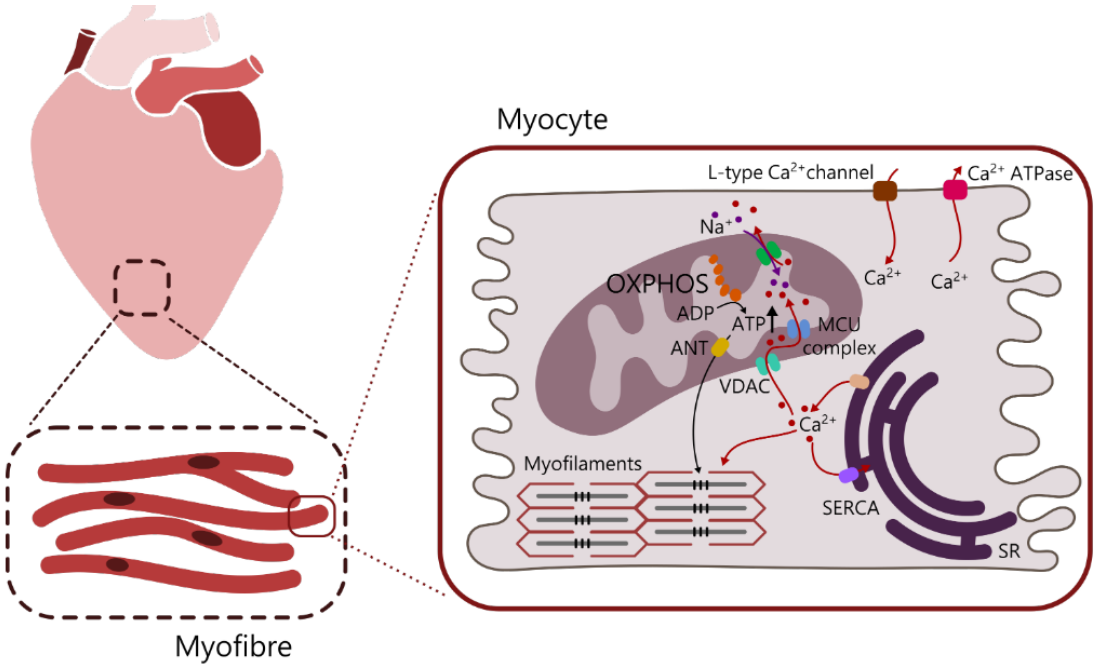
Figure 6. Cardiovascular benefits of regular exercise and some pharmacological interventions.


Intrinsic Factors
(Epi) Genome
Intrauterine and Early Life
Behavioral Factors
Physical Inactivity
Smoking and Alcohol consumption
Poor Diet
Environmental Factors
Air pollution and living conditions
Physiological Factors
Diabetes and raised blood sugar levels
Obesity and raised blood lipids
Inflammation and Thrombosis
Hypertension
Cardiovascular Risk Factors
Cardiovascular Diseases
ischemic heart disease, stroke, heart failure, peripheral arterial disease, rheumatic heart disease

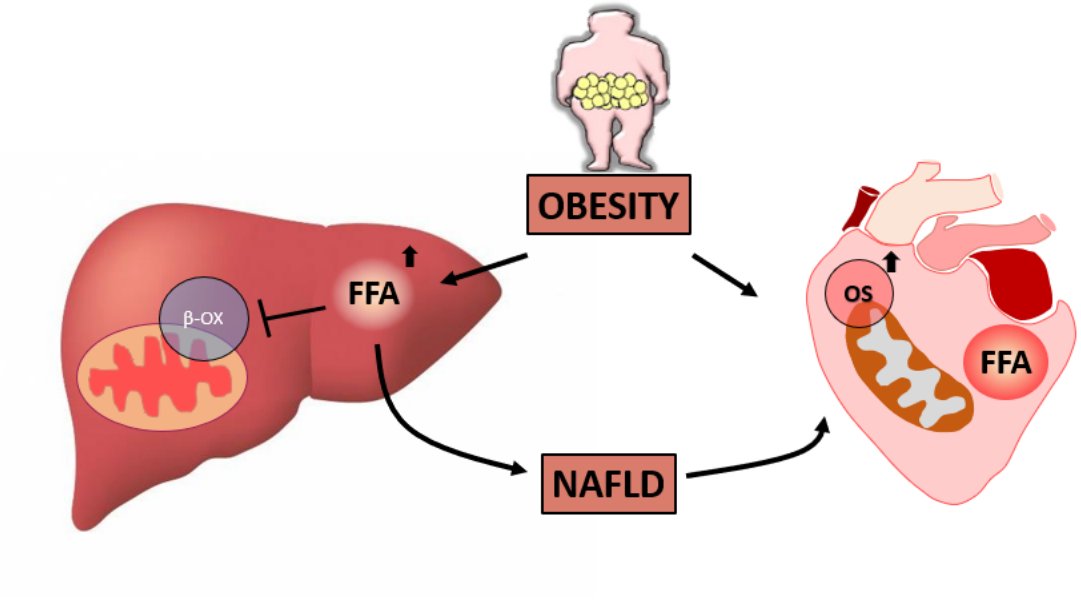
1


Inflammation
Hyperglycaemia
• ER stress
• ROS
• Inflammation
• Apoptosis
Hypertension
Insulin resistance
• OS
• Hypertension
• Inflammation
Dyslipidaemia
• ROS
• OS
• LDL
• Inflammation
Cardiovascular Risk

1


Myocyte
L-type Ca2+channel
Ca2+ ATPase
Na+
OXPHOS
ADP
ATP
ANT
MCU
complex
VDAC
Ca2+
Ca2+
Ca2+
Myofilaments
SERCA
SR
Myofibre

1



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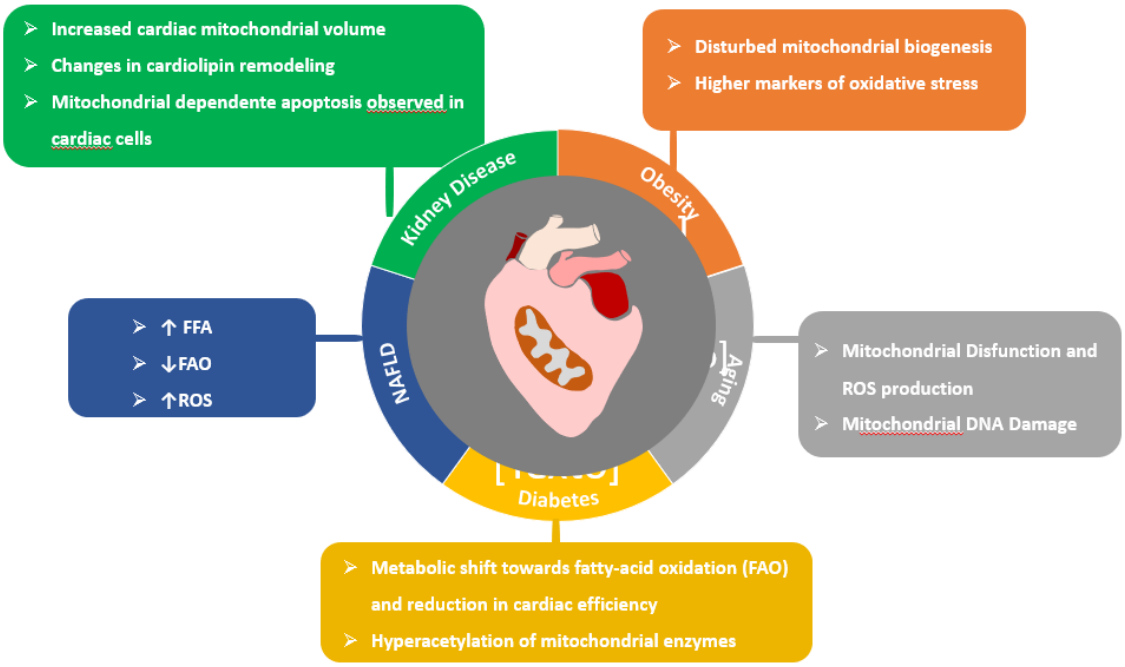
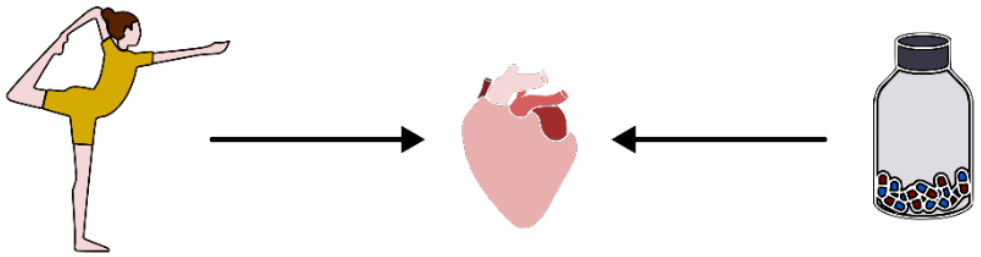

Increased cardiac mitochondrial volume
Changes in cardiolipin remodeling
Mitochondrial dependente apoptosis observed in cardiac cells
Disturbed mitochondrial biogenesis
Higher markers of oxidative stress
Kidney Disease
Obesity
NAFLD
Aging
Diabetes
↑ FFA
↓FAO
↑ROS
Mitochondrial Disfunction and ROS production
Mitochondrial DNA Damage
Metabolic shift towards fatty-acid oxidation (FAO) and reduction in cardiac efficiency
Hyperacetylation of mitochondrial enzymes

1



Regular Exercise

- ↑ Vascular endothelial function (↑eNOS, ↑NO)
- ↑ Protection against ischemia-reperfusion injury
- ↑ Mitochondrial Biogenesis (↑PGC1α, ↑musclin)
- ↑ Mitochondrial capacity to retain calcium ions (↓susceptibility to Ca^{2+}-induced mPTP opening)
- ↑ Proteins involved in FAO

Pharmacological Interventions

CoQ_{10}: antithrombotic and antioxidant properties; ↑endothelial function

$MitoQ_{10}$: antioxidant properties; protection against hypertension, cardiac hypertrophy and ischemia-reperfusion injury

ACE inhibitors: ↑mitochondrial biogenesis

Antidiabetic drugs: ↓mitochondrial ROS production; ↑antioxidant defenses; ↓inflammation

1